Method Review by the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM)

ICCVAM was created as an ad-hoc committee in 1994 and became a standing Committee in 2000. ICCVAM has neither scientific staff nor labs; its participation in validation of alternative methods has largely been as a member of an OECD or ECVAM review committee and/or by convening its own review panels to review methods that have been validated elsewhere.

In its Biennial Progress Report 2008-2009 and in correspondence from Secretary Kathleen Sebelius, ICCVAM states that "***ICCVAM has contributed to the approval or endorsement of 33 alternative safety testing methods by Federal regulatory agencies since its establishment in 1997***".[1] To investigate this claim, PETA reviewed each of the 33 methods. They are listed below, along with a factual timeline for how they were nominated, reviewed, validated, endorsed, or recommended.

The 33 methods can be categorized based on ICCVAM level of involvement: ***substantial effort 12% (4 methods), reviewed others' efforts 42% (14 methods), and little or no effort 45% (15 methods)***. Therefore, of the 33 methods ICCVAM lists as having contributed to, it did so significantly only for four: the original LLNA protocol and performance standards, Corrositex skin barrier method for corrosion testing and performance standards, and the BCOP and ICE excised eye methods for corrosion testing. ICCVAM has reviewed cytotoxicity methods for setting starting doses for lethal toxicity tests in animals but there has been no follow up with regard to replacement, even after a decade. *Overwhelming evidence shows ICCVAM* ***has not substantially contributed to, and cannot take credit for, more than 87% of the list below.***

	Method	Review Process	ICCVAM Contribution
1	**Murine Local Lymph Node Assay for skin sensitization**	The Murine Local Lymph Node Assay (LLNA) is a reduction (4 -5 mice vs. 10 – 20 Guinea pigs) and refinement over the Guinea pig-based tests. The LLNA was nominated by industry in 1997. Later that year ICCVAM reviewed the validation material supplied by the sponsors in 1998 and issued a report describing the "usefulness and limitation's" of the method in 1999. ICCVAM recommended the LLNA to US agencies in 1999 as an alternative for Guinea pig tests for allergic contact dermatitis.[2] It was adopted in 2002 as TG 429 by OECD.	Substantial review, recommendations and creation of performance standards
2	**Up-and-down procedure for acute oral toxicity**	The Up Down Procedure (UDP) has been an OECD Test Guideline since 1998 (TG 425); validation studies were supplied by industry, academia, and European Government. US Environmental Protection Agency (EPA) organized a technical task force to revise UDP and in 1999 asked ICCVAM to conduct an independent peer review panel evaluation of the revised UDP.[3] The peer review panel concluded that the revised UDP was acceptable as an alternative to the traditional LD50 for assessing acute oral toxicity. ICCVAM made recommendations to US agencies following OECD acceptance of TG 425. A final revised version of TG 425 was accepted by OECD as a *replacement* for TG 401 in 2006.	Review of update
3	**Fixed dose procedure for acute**	Following a validation study of this method by the	Minor if any

	oral toxicity	European Commission and UK government, the Fixed Dose Procedure (FDP) was accepted as an alternative to TG 401 by OECD as TG 420 in 1992. This method was accepted by OECD as a replacement for the traditional LD50 in 2001, along with the Acute Toxic Class method.[4] ICCVAM participated in the revision of this TG by OECD by providing comments in the same way as other stakeholders.	
4	**Acute Toxic Class method for acute oral toxicity**	Following the 1995 publication of an international validation study sponsored by the German Government, the Acute toxic class (ATC) method was adopted by OECD as TG 423 in 1996 as an alternative to TG 401. In 2001, it was adopted as a replacement for TG 401. ICCVAM participated in the review in the same manner as other stakeholders.	Minor if any
5	**EPISKIN™ *in vitro* human skin model skin corrosivity test**	A validation study for EPISKIN™, Rat TER, and CORROSITEX® was managed and funded by ECVAM from 1996 - 1997. ECVAM Scientific Advisory Committee (ESAC) recommended EPISKIN™, Rat TER, and CORROSITEX® in 1998; and they were accepted as OECD Test Guidelines: EPISKIN™ under TG 431 in 2004, Rat TER as TG 430 in 2004, and CORROSITEX® as TG 435 in 2006. ICCVAM received a nomination for CORROSITEX® from industry (InVitro International, Inc.) in May 1998, ***after*** validation was completed by ECVAM. ICCVAM reviewed the data package from ECVAM which included 163 chemicals with correlating *in vivo* rabbit data and made recommendations to US agencies in 1999.[5] EpiDerm™ was validated by ECVAM in a catch-up validation by comparison to Performance Standards developed by ICCVAM and recommended by ESAC in 2000. EpiDerm™ was accepted by OECD in 2004 under TG 431. SkinEthic™ underwent an independent catch-up validation (sponsored neither by ECVAM nor by ICCVAM), was endorsed by ESAC in 2006 and accepted by OECD under TG 431.	Review and recommendation of Corrositex®, creation of performance standards
6	**Rat TER *in vitro* skin corrosivity test**		
7	**CORROSITEX® *in vitro* membrane barrier skin corrosivity test**		
8	**EpiDerm™ *in vitro* human skin model skin corrosivity test**		
9	**SkinEthic™ *in vitro* human skin model skin corrosivity test**		
10	**3T3 NRU phototoxicity test for skin photo-irritation**	ECVAM held a workshop in 1993 on *in vitro* phototoxicity testing and international ECVAM-sponsored validation studies were underway the same year. Following completion of the validation study, these methods were endorsed by ECVAM and ESAC in 1997 (method 10) and 1998 (method 11). ICCVAM participated in the OECD review of these methods in the same way as other stakeholders. In May of 2003, FDA Center for Drug Evaluation and Research (CDER) issued a guidance document on photo safety testing; ICCVAM was not	Minor if any
11	**3T3 NRU phototoxicity test: application to UV filter chemicals**		

		acknowledged as having participated.	
12	***In vitro* dermal absorption methods**	OECD officially adopted TG 427 (*in vivo* dermal absorption) and TG 428 (*in vitro* dermal absorption) in April 2004. It is not clear how or if ICCVAM contributed to this effort; validation studies were not conducted by ECVAM or ICCVAM. In 2004 van de Sandt et al. compared *in vivo* and *in vitro* data (both inter- and intra- laboratory) in a quasi-validation exercise. They concluded that *in vitro* methodology for assessing skin absorption was robust.[6] This study was financially supported by the Fifth Framework Program of the European Commission.	Minor if any
13	**Use of humane endpoints in animal testing of biological products**	The endpoint for potency testing of biological products is death. In this method refinement, the animals may be spared a day or two of suffering (at the ***end*** of an experiment) but their pain and distress is ***not significantly reduced***. This initiative is simply an official acceptance by Center Veterinary Biologics (CVB) of a definition for "humane endpoint", as outlined in a CVB guidance document.[7] ICCVAM may have contributed to the language through one of their members from United States Department of Agriculture (USDA) but that information is not forthcoming. ECVAM held a workshop on quality control in rabies vaccines in April 2002 and ICCVAM has a vaccine-related workshop planned for September of 2010.	Wording revision resulting in minor refinement
14	**Rabies vaccine, humane endpoints**		
15	**Uterotrophic bioassay in rodents: a short-term screening test for estrogenic properties**	This method has been in use since the 1930s, and was validated for regulatory purposes through OECD beginning in 1998, and adopted as TG 440 in 2007. ICCVAM participated in review of this TG by submitting comments to OECD, in the same way as other stakeholders, including ICAPO.[8]	Minor if any
16	**Bovine corneal opacity and permeability test method (BCOP) to identify severe eye irritants/corrosives**	US EPA nominated 4 *in vitro* methods for evaluation by ICCVAM in October 2003 (BCOP, ICE, Hen's Egg Test-Chorioallantoic Membrane assay, and isolated rabbit eye). ICCVAM requested validation data from the public twice (both in 2004 and 2005). ICE validation data came from industry (Prinsen 1993, 2000, and 2005 and Balls 1995).[9] BCOP data came from eight publications, submissions, or study reports; both industry and ECVAM studies were included.[10] After reviewing these data, expert panel summary, public comments, etc. ICCVAM prepared the Test Method Evaluation Report[11] and recommended BCOP and ICE to US agencies as screening tests to identify substances as ocular corrosives and severe irritants for use in a tiered approach. ESAC agreed to these recommendations also in 2007. In 2009, BCOP was adopted as OECD TG 437 and ICE as TG 438.	Substantial review and recommendations
17	**Isolated chicken eye test method to identify severe eye irritants/corrosives**		
18	**Acute toxicity *in vitro* starting dose procedure, 3T3 cells**	These methods are simply used to determine a ***starting dose for animal tests***; there is a reduction of animals used for range finding but does not reduce the animals used in further studies and is not in itself	Initial review, recommend for *starting doses* after *nine* years, no
19	**Acute toxicity *in vitro* starting dose procedure, NHK cells**		

		a regulatory test. In response to a request from EPA OPPTS in 1999, ICCVAM initiated a review of these methods (18, 19).[12] ***Three years*** after the nomination, validation studies were underway, supported by ECVAM and NIEHS. Another ***six years*** passed before ICCVAM made recommendations to US agencies in 2008. ICCVAM has failed to further investigate the applicability of these methods for acute toxicity testing itself.	follow-up with regard to replacement
20	**ELISA test for batch potency testing of erysipelas vaccines (refinement: antibody quantification)**	It does not appear that ICCVAM contributed to this effort (ICCVAM's website says they "coordinated agency consideration"). Validation studies were handled in Germany at the Paul Ehrlich Institute, initiated by ECVAM. In 2002, ESAC issued a statement accepting the scientific validation of this method. Agencies review on a case by case basis whether *in vitro* or *in vivo* data is sufficient for batch potency testing. (This is a swine vaccine, relevant to a single agency, the USDA).	None
21	**Relevance of the target animal safety test for batch safety testing of vaccines for veterinary use**	Target animal batch safety testing is only validated in the EU. It is CVMP-related and is not even allowed in the US; ICCVAM could not have initiated this action. In 2002, ESAC unanimously decided that target animal safety batch testing requirements should be waived for routine batch release.[13]	None
22	**ELISA test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)**	Both human tetanus methods (22, 23) are ECVAM-validated and FDA has not verified its acceptance of either method.[14]	None
23	**ToBI test for batch potency testing of human tetanus vaccines (refinement: antibody quantification)**		
24	**Human whole blood/interleukin-1β *in vitro* pyrogen test**	These five *in vitro* pyrogen tests are very similar methods for the same endpoint and were reviewed at the same time.[15] [16] ECVAM held a workshop on novel pyrogen tests in January 2000, prioritizing the need for new, more specific tests that would detect all pyrogens and support the 3R's. Pre-validation and validation studies were conducted by ECVAM and by 2005 they nominated these five methods to ICCVAM. ICCVAM requested the ESAC peer review report but it was not publicly available so they utilized background review documents from ECVAM. The review process was slow because ICCVAM insisted on direct comparisons of *in vitro* data with both Rabbit Pyrogen Test and Bacterial Endotoxin Test data. ICCVAM convened a peer review panel in 2007 that rejected these methods based on a scientifically flawed review. While	Re-evaluation of ECVAM review, resulting in a three-year delay in recommendation for use in US
25	**Human whole blood/interleukin-1β *in vitro* pyrogen test: application of cryopreserved human whole blood**		
26	**Human whole blood/interleukin-6 *in vitro* pyrogen test**		
27	**Human peripheral blood mononuclear cell/interleukin-6 *in vitro* pyrogen test**		
28	**Monocytoid cell line Mono Mac 6/interleukin-6 *in vitro* pyrogen test**		

		ECVAM were able to recommend these methods within one year (2006), and even though a member of ICCVAM observed the ECVAM review, it took three years for ICCVAM to endorse them in 2008. In contradiction to ICCVAM's own sponsored peer review, ICCVAMs eventual conclusions were identical to those of ECVAM.	
29	**Inhalation toxicity - acute toxic class method**	Although ICCVAM hosted a workshop on acute chemical safety testing in February, 2008 (along with JaCVAM and ECVAM), reference materials for the workshop did not include OECD TG 403 (the acute inhalation method in use at the time) and the workshop agenda did not include the topic of acute toxic class for inhalation toxicity.[17] ICCVAM may have participated in the review process as a stakeholder. OECD TG 436 was adopted in 2009 and ICCVAM simply approved via US representatives to OECD.	Minor if any
30	**Hershberger bioassay in rats: a short-term screening assay for (anti) androgenic properties**	US EPA Endocrine Disrupter Screening and Testing Committee nominated the Hershberger to OECD for validation (for specific use in Tier I EDSP battery) and US EPA laboratories took the lead in validation exercises. OECD TG 441 was adopted in 2009. ICCVAM participated in the OECD review by providing comments in the same way as other stakeholders, including ICAPO.[18]	Minor if any
31	**Stably transfected human estrogen receptor-α transcriptional activation assay for the detection of estrogenic agonist-activity of chemicals**	In 1998 OECD initiated a high-priority activity to revise existing and develop new screening tests to determine endocrine disrupting potential. In 2000 EPA nominated this method along with other *in vitro* endocrine disruptor assays to ICCVAM for consideration. An expert panel was convened to review existing literature and determined that none of the methods were adequate.[19] Development and validation of this particular assay was pursued by Japan, and the validation was completed through the OECD, and accepted as OECD TG 455 in 2009. ICCVAM participated by providing comments in the same way as other stakeholders, including ICAPO.[20] There are two *in vitro* estrogen receptor assays under evaluation by ICCVAM: the LUMICELL® ER transcriptional activation assay, nominated in 2005, and the CertiChem MCF-7 Cell Proliferation assay, nominated in 2006, both of which are still in review.	Minor
32	**Updated LLNA protocol (requires 20% fewer animals)**	Both of these methods are refinements of the LLNA test. In 2006 the developers of LLNA published a retrospective analysis of the literature and concluded that a reduced version of LLNA could be used as a screening test to distinguish sensitizers from non-sensitizers.[21] Based on this paper, ECVAM initiated action in 2007 and by April of the same year ESAC issued a statement endorsing this method. In	Development of performance standards, secondary review of ECVAM review resulting in a three-year delay in recommendation
33	**Reduced LLNA protocol (requires 40% fewer animals by using only the high dose group)**		

		response to a nomination by the Consumer Products Safety Commission (CPSC), ICCVAM convened a peer review panel who met in 2008 and 2009 to review several versions of LLNA tests, including both updated and reduced protocols, among others. In 2008, ECVAM and ICCVAM independently developed LLNA Performance Standards. Subsequently, the two groups harmonized their reports with respect to essential test method components, minimum list of reference chemicals, and accuracy and reliability values. A background review document on the reduced method was published in 2009.[22] ICCVAM made affirmative recommendations to US agencies in 2010, specifically stating that the rLLNA was properly validated for use in distinguishing sensitizers from non-sensitizers in cases that do not require dose-response information. ECVAM makes no mention of the updated LLNA, and it was not specifically recommended to US agencies by ICCVAM, presumably because the reduced method uses fewer animals than updated LLNA. Revisions to OECD TG 429 are pending.	for use in US.

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